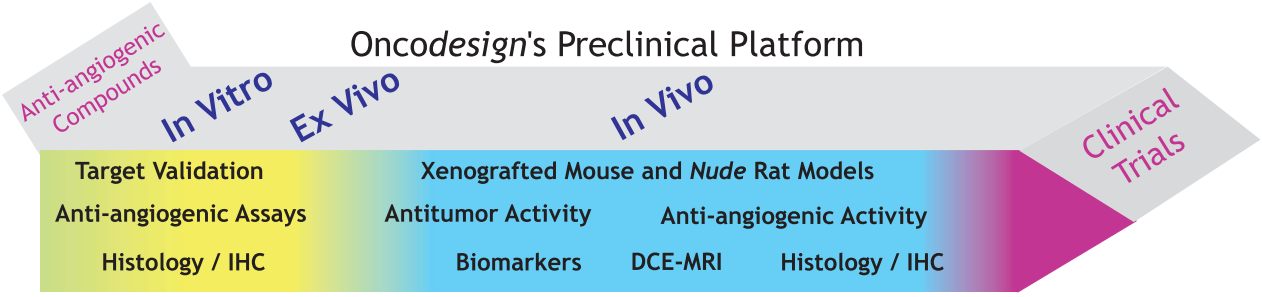
Assessment of Anti-Angiogenic Therapies

• IMAGING • BIOMARKERS • PRECLINICAL • PHARMACOLOGY • ONCOLOGY •

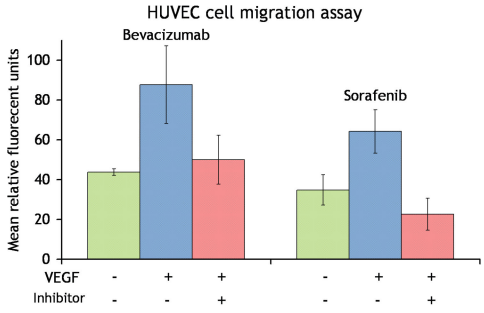
How to Address Angiogenesis and an Antitumor Effect in a Translational Setting



The discovery that tumor progression is sustained by neo-angiogenesis has led to the development of successful therapies targeting the formation of blood vessels, such as Avastin®. Oncodesign's models, imaging technology and comprehensive strategy provide preclinical and translational data for clinical use, creating a significantly better opportunity for clinical success.

Key Benefits

- *In vitro* effect on endothelial cells and blood vessel formation
- *In vivo* evaluation of antitumor activity, determination of the biological active/optimal dose, PK/PD, resistance diagnostic, selection of responder subpopulations, therapy combinations
- Translational imaging biomarkers by MRI (Magnetic Resonance Imaging)
- Histologic validation of anti-angiogenic effects



In Vitro Angiogenesis Assays

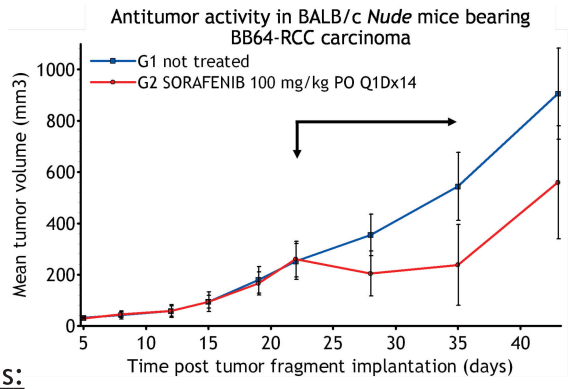
Target validation:
- High-throughput cell line screening
- Flow cytometry, immuno-histochemistry

Analysis of anti-angiogenic potency:
- Migration assay
- Invasion assay

In Vivo Antitumor Activity

Xenografted mouse models:
- A673 rhabdomyosarcoma cells express a high level of VEGF (model used to validate Avastin®)
- BB64-RCC renal carcinoma: key pathology for the clinical evaluation of anti-angiogenic drugs

Xenografted *Nude* rats:
- Calu-6 squamous cell carcinoma (good perfusion and sensitive to anti-angiogenic compounds)
- MDA-MB-231, HCT 116, U-87 MG...



Oncodesign's expertise with anti-angiogenic compounds:

Anti-VEGF (bevacizumab), multi kinase inhibitors (sorafenib, sunitinib, anti-VEGFR, anti-FGFR, anti-Tie2...), integrin inhibitors (anti αVβ3, anti α5β1), anti CD44, hormonal therapies, and vascular targeting agents.

In Vivo Tolerance and Pharmacokinetics

- Acute toxicity (MTD)
- Cumulative tolerated dose (MTTD)
- Biodistribution
- Bioavailability

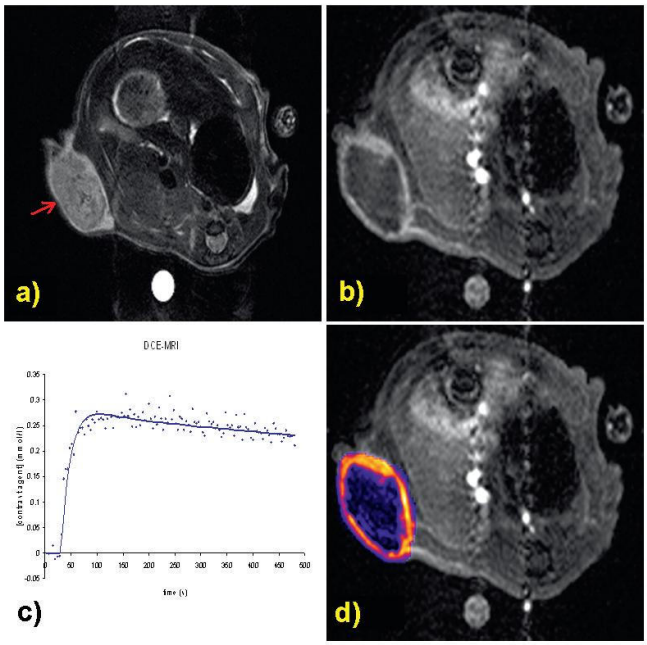
Translational Imaging Biomarkers by MRI

Applied to angiogenesis, imaging provides a spatial and temporal mapping of physiologic parameters such as perfusion and tumor micro-vessels permeability to evaluate the heterogeneity within a tumor or between tumors. The leading imaging technique to quantitatively asses the effect of novel compounds on tumor vasculature is Dynamic Contrast-Enhanced Magnetic Resonance Imaging (DCE-MRI).

At Onco*design*, we have been using DCE-MRI for ten years in studies in which it has played a central role in the Go/No Go decision. We have performed a total of more than 1200 imaged animals (rats and mice): optimized protocols, validated animal models and powerful image analysis tools, together with our well-known ability to design and conduct research projects, will help you to build the best translational study to support clinical trials with your compound.

DCE-MR Imaging protocol:

- Morphological imaging to estimate tumor volume (a)
- Dynamic contrast-enhanced (DCE) MRI
  - baseline image acquisition
  - bolus injection of Gadolinium-based contrast agent
  - dynamic follow-up of contrast agent uptake in the tumor (b,c)
- Quantitative image analysis using in-house developed software.
  Endpoints: Initial Area Under the Curve (IAUC), Ktrans tumor mapping (d)



Example images from a DCE-MRI protocol of a subcutaneously grafted Calu-6 tumor (a, red arrow) and voxel mapping of the contrast agent transfer constant K^{trans}

Additional Imaging biomarkers:

Vessel Size Index (VSI)
Blood Volume (BV)
Apparent diffusion coefficient (ADC)

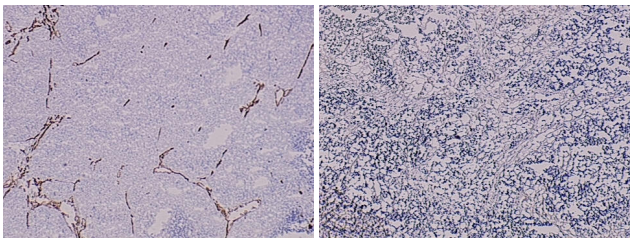
Applications:

- Characterization of tumor perfusion and microvascular permeability
- PK/PD relationship
- Biological active dose identification
- Proof of concept support / mechanism of action
- Study of potential synergistic therapy combinations
- Biomarker for antitumor efficacy

Benefits:

- Serial tumor follow-up, Non invasive / well tolerated
- Early evaluation of the anti-angiogenic effect
- Translational biomarker included in Phase I/II clinical trials

Histological Validation of Tumor Vascularization



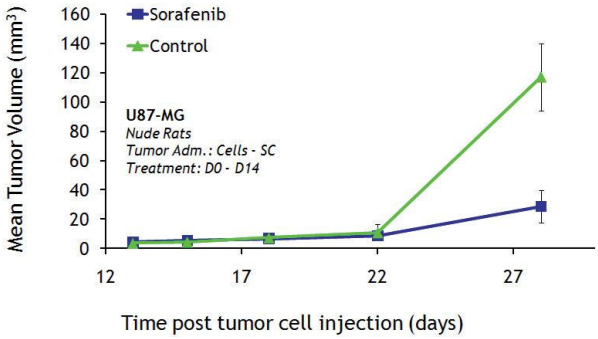
CD31 staining of untreated A673 tumor (left) or treated with bevacizumab (right)

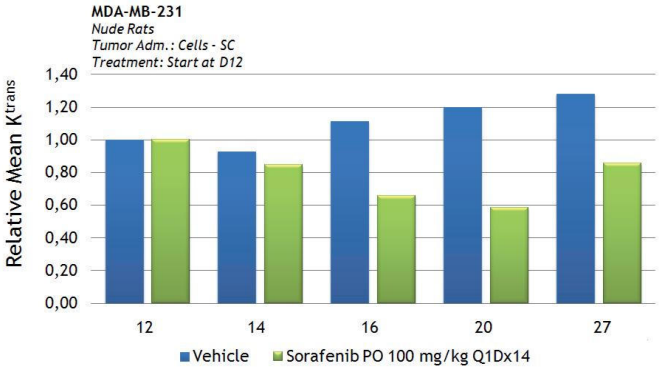
- Immuno-histochemistry and semi-quantitative analysis:
  - CD31 or RECA-1 staining to analyze mean vascular density and vessel distribution in the tumor
  - CD31/SMA co-staining for neo-angiogenesis and vessel maturity evaluation
- Evaluation of hypoxia: CA IX, Pimonidazole
- Functional assay to characterize vascular permeability:
  - fluorescent microscopy of cryosections after *in vivo* injection of Hoechst dextran-FITC or lectin-FITC
  - quantitative measurement of the number/size of vessels (dextran, lectin) & vascular permeability (Hoechst)

Drug Resistance Monitoring

Biomarkers of tumor escape are needed to identify the emergence of resistance to antiangiogenic agents:

- Early detection of tumor escape to treatment
- Identification of the timing and the mechanism of drug resistance
- Identification of additional targets on tumor cells / tumor microenvironment
- Development of potential synergistic therapy combination to combat resistance

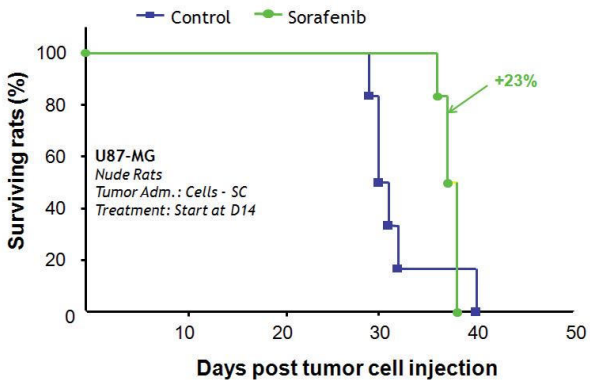




MRI allows to monitor drug resistance, bringing both morphological data (top, tumor growth of U-87 MG-bearing rats daily treated with Sorafenib for two weeks) and functional data (left, transitional decrease of K^{trans} in MDA-MB-231 tumors in *Nude* rats treated with Sorafenib) - while Sorafenib induces a moderate effect on the survival (bottom).

<u>Multiparametric imaging allows to address the complexity of antiangiogenic therapies:</u>

- Reduction in tumor perfusion (K^{trans}, DCE-MRI, top)
- Reduction in vessel density (IHC)
- Increase of intratumoral ADC (DW-MRI)
- Increase of metabolite-associated glycolysis (lactate, choline, taurine) (^{1}H-MRS)
- Transient effects on tumor growth (morphological T2w-MRI, top right)
- Limited effects on overall survival (bottom right)



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